63-4-5

NATURAL TOXIC AND NARCOTIC COMPOUNDS FROM PLANTS

Contract Nr. DA-91-591-EUC-2580

N A T U R A L T O X I C A N D N A R C O T I C C O M P O U N D S F R O M P L A N T S

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Contract No. DA-91-591-EUC-2580

F i n a l T e c h n i c a l R e p o r t

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Abstract

Work was continued on the chemistry of plant constituents which had been isolated from plants showing toxic or other pharmakologically interesting properties (see Final Technical Reports, Contract Nrs. DA-91-591-EUD-1493 and -1799). The following results have been obtained:

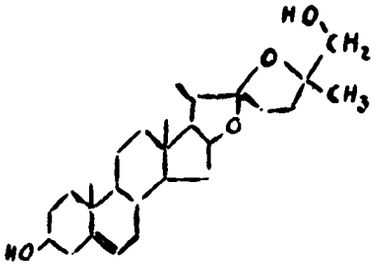
Daphne mezereum (thymeleaceae)
Daphnoretin, $C_{19}H_{12}O_7$, was identified as 6-methoxy-7-hydroxy-3,7'.dicumaryl ether and its structure confirmed by synthesis of its methyl ether. It also occurs as ß-glucoside. Tests for possible effects on blood coagulation were negative. Experiments to isolate the skin irritating material contained in the seeds in pure form were unsuccessful. From the active fraction an unsaturated hydroxy ester $C_{29}H_{30}O_8$ was isolated pure which was toxic to mice but showed no skin irritating properties.

Ipomoea fistulosa (convolvulaceae)
The toxic ester glycosides found in the leaves were found to have a molecular weight near 20,000 with a broad distribution curve. Alcaline hydrolysis gave among other products a straight chain monohydroxy acid $C_{11}H_{22}O_3$ which was also obtained on oxydation of the main aglycone $C_{15}H_{28}O_4$ (I) on oxydation with hypoiodite or periodate under alcaline conditions. For I the structure of a 3,5,x-trihydroxy pentadecanoic acid δ-lactone is assumed from the data of lactone tiration, IR-spectrum, and oxydation to a ß-keto lactone with chromic acid.

Piptadenia peregrina
Three tryptamine bases have been isolated from the bark and been identified as N-methyltryptamine, 5-methoxy-N-methyltryptamine, and 5-methoxy-N,N-dimethyltryptamine.

Solanum sisiimbrifolium (Solanaceae)

Further investigation of the root saponin showed that the original sapogenin is one which contains a five membered ring F in the spirostanol side chain: During the acid hydrolysis this rarranges to the normal six membered form. In addition two products were isolated containing a Δ^3 double bond as result of a dehydration in position 3 of the steroid skeleton.



Symplocos celastrinea (symplocaceae)

Alcaloid A, $C_{19}H_{21}O_4N \cdot CH_3OH$, was identified as 3,6-dihydroxy-2,5-dimethoxy-aporphine by comparison of its methochloride with an authentic sample of lyurifilin chloride.

For alcaloid C, $C_{17}H_{17}O_2N$, the structure of 5-hydroxy-6-methoxy-N-desmethyl aporphine is proposed and experiments leading towards its synthesis described.

Asclepias mellodora (asclepiadaceae)

This toxic plant from Argentina was shown to contain heart poisons of the cardenolide type.

Introduction

This report is to be regarded as continuation of Final Technical Report Contract No. DA-91-591-EUC-1799 (in the following referred to as previous FTR) and of Final Technical Report Contract No. DA-91-591-EUC-1493. In this research program as yet uninvestigated plants were to be investigated for toxic or pharmacologically active compounds which were then to be isolated and their structure determined. In all 28 species have been collected in Brazil and Paraguay. They had been chosen on the basis (information by local botanists) that compounds of interest could be expected in these plants. Of these about 6 were found to contain material suitable for further studies and were investigated more closely after the necessary experimental details had been worked out. With the others no effects of interest were observed, either because the active material did not survive the drying and isolation procedures, or the plants could not be collected at the right season, or the test animals used (generally mice) were not susceptible.

Work on the following plants was continued during the period covered by this report:

D a p h n e m e z e r e u m (Thymeleaceae)
I p o m o e a f i s t u l o s a (convolvulaceae)
P i p t a d e n i a p e r e g r i n a (Leguminosae)
S o l a n u m s i s i i m b r i f o l i u m (solanaceae)
S y m p l o c o s c e l a s t r i n e a (symplocaceae)

Preliminary results have already been given in the previous FTRs.

In addition a toxic plant from Argentina, A s c l e p i a s M e l l o d o r a (asclepiadaceae) was investigated. From the paper chromatographic results it could be inferred that the active compounds are probably heart poisons of the cardenolid type which are widely distributed in this plant family and which were therefore not investigated further at present.

Daphne mezereum (thymeleaceae)
(see previous FTR p.4)

Studies of the skin irritating material contained in the seeds were hampered by the lack of seeds in 1962 and by the lack of seeds in 1962 and by the difficulties of isolating pure compounds with skin irritating properties from the active fractions. Only resinous material still impure was obtained after repeated column and preparative thin layer chromatography. The most active fractions gave a strongly positive test for hydroxamic acids after treatment with hydroxylamine and alcali, indicating ester or lactone groups. From the position of the carbonyl bands in the infrared spectrum the presence of an o-hydroxyester (chelation) was indicated.

As has already been mentioned in Final Technical Report, Contract Nr. DA-91-591-EUC-1493 OI-4665-60 a crystalline compound (Compound A), m.p. 265-8, was isolated from the fractions containing the skin irritating material. Elemental analysis indicated the formula $C_{29}H_{30}O_8$. Functional groups are hydroxyl (non phenolic), keto and ester groups, and olefinic double bonds. Hydrolysis with dilute alcali does not result in simple saponification but leads to several rearranged products as can be seen from a comparison of the UV-spectra of Compound A and its degradation products. Compound A has no skin irritating properties but is toxic to mice. Intraperitoneal injection of a 2.5 % suspension in 33 % polyethylene glycole lead to death within 16 hours at 500 mg/kg. At 150 mg/kg paralysis of the hind legs was observed together with occasional spasms.

The structure of daphnoretin, a cumarin derivative $C_{19}H_{12}O_7$, which had also been found in Daphnopsis racemosa, has been confirmed by synthesis of its methyl ether. 3-bromo-6,7-dimethoxy-cumarin and the potassium salt of 7-hydroxy cumarin were hea ted with copper powder to give the desired product in 60 % yield. Daphnoretin is thus 6-methoxy-7-hydroxy-3,7'-dicumaryl ether.

From the more polar fractions a glycoside daphnorin was isolated, which was found to consist of one mole of glucose and one mole of daphnoretin. This glycoside was tested for toxic effects with mice. No effects could be observed at 100 mg/kg. Further tests with rabbits for possible effects on blood coagulation (inhibition of prothrombin synthesis by vitamin K antagonism in analogy to dicumarol) were negative.

Ipomoea fistulosa (convolvulaceae).
(see previous FTR, p. 5)

Fraction B of the toxic glycosides found in the leaves was investigated further. Molecular weight determinations by osmotic pressure measurements gave a value of 11,000 or 26,000 depending on the pore size of the osmometer membrane. Investigations with the ultracentrifuge showed a broad almost symmetrical distribution curve with a single maximum. From the maximum a sedimentation constant of 2.61 Svedberg units was calculated which corresponds to a molecular weight of 23,000 assuming spherical particles.

Like jalap resin glycoside B, which is almost insoluble in water can be solubilized with sodium cholate to give a clear aqueous solution with strongly hemolytic properties. Glycoside B in this form is also toxic to mice (lethal dose about 500 mg/kg p.o.) At a concentration of 0.25 % sodium cholate will solubilize about 20 times its weight of glycoside B.

Hydrolysis of the glycoside with barium hydroxide at room temperature was found to give in addition to the substances found previously (glycosidic acids with equivalent weight 1,100, tiglic and isovaleric or α-methyl-butyric acid) an acid $C_{14}H_{28}O_8$ (formula calculated from the composition of its S-benzyl-isothiuronium salt). This acid could be extracted from the acidified hydrolysis mixture with ethyl acetate after the more lipophilic fatty acids had been removed with ether. On hydrolysis with acids an unidentified hydroxy-acid and a sugar which corresponded to one already detected by paperchromatography in hydrolysates of the whole glycoside. It behaves similar to rhamnose and fucose but gives a different reaction with anthrone/sulfuric acid.

Treatment of the ether soluble acids with petroleum ether pecipitated hydroxylated fatty acids from which one could be isolated pure. This acid was shown to be identical (m.p., IR-spectrum, thin layer chromatography) with a monohydroxy-acid $C_{11}H_{22}O_3$, m.p. 100-102 obtained from the dihydroxy-lactone $C_{15}H_{28}O_4$ on degradation with alcaline hypoiodite or alcaline periodate. This lactone (genin D) was the main aglycon obtained on acid hydrolysis of the glycosidic acids.

Genin D had been found to contain two free hydroxyl groups by analysis of its p-nitrobenzoate and a δ-lactone ring by lactone titration and IR-spectroscopy. It gave a positive iodoform test and could be oxidised to give a diketone which gave a positive enole reaction with $FeCl_3$. In addition a monoketone was isolated from the oxydation mixture which still contained a hydroxyl group and which did not react with $FeCl_3$. An explanation of these results by assuming the structure CH_3-CHOH-CH_2CHOH-CH_2- in the side chain of a saturated lactone could not be confirmed by further experiments. Quantitative determination of the iodoform formed on oxydation with hypoiodite showed that the amount was much too small to have arisen from a CH_3-CHOH group. Furthermore it was found that the UV-spectrum of the diketone in acid and alcaline solution closely resembled that of an ß-keto ester or ß-keto lactone instead of a ß-diketone.

The degradation product obtained under the conditions of the iodoform test is an acid $C_{11}H_{22}O_3$ m.p. 100-102° which was also obtained on oxidation with alcaline periodate. In both cases the yield was 98-99 %. No reaction was observed with periodate under neutral or acid conditions. The acid formed is a straight chain monohydroxy acid whose methyl ester decomposed in the mass spectrometer like an α-hydroxy ester (no parent peak, loss of 59 mass units due to removal of the CO-OCH_3 group). Attempts to confirm the α-position of the hydroxyl group by chemical reactions were unsuccessful however. Experiments to degrade the hydroxy acid via its keto analogue are currently in progress. The assumption of a branched chain for genin D, which resulted from C-methyl values (Kuhn-Roth oxydation) corresponding to two methyl groups could not be verified by NMR-spectroscopy. These high results are probably caused by hydrolysis and decarboxy-

lation of the β-keto lactone formed on oxydation.

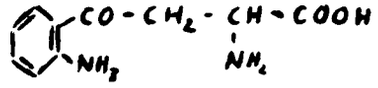
P i p t a d e n i a P e r e g r i n a (leguminosae).
(see previous FTR p. 12)

The bark of this tree had been found to contain about 0,15% of basic material. The main components were similar to but not identical with N,N-dimethyltryptamine and bufotenine which had been reported to be present in the seeds of this plant.

Countercurrent distribution over twelve steps between methylene chloride and phosphate puffer pH 4.8 separated strongly and weakly basic material. Column chromatography of the former on deactivated alumina (III) gave two main fractions. The first gave colorless crystals from benzene/cyclohexane, m.p. 67-8°, analysing for $C_{13}H_{18}N_2O$. It was identified as 5 - m e t h o x y - N,N- d i m e t h y l - t r y p t a m i n e by comparison with an authentic sample prepared from bufotenin with diazomethane.

From the second fraction a compound $C_{12}H_{16}N_2O$, 1 O-CH_3, 1 N-CH_3, m.p. 99-102, was obtained on crystallisation from benzene. Analytical data, UV-spectrum and m.p. of it picrate, hydrochloride and p-toluenesulfonyl amide indicated that it was 5- m e t h o x y- N - m o n o m e t h y l t r y p t a - m i n e . Its methoiodide was identical with the methiodide from 5-methoxy-N,N-dimethyltryptamine. Examination of the second fraction by paper chromatography revealed the presence of a third compound which could be separated from the second by column chromatography of the toluenesulfonyl amides. The toluenesulfonylamid was identical with an authentic sample of N-methyl-N-p-toluensulfonyl tryptamine, so that the third compound is N - m e t h y l t r y p t a m i n e. These three tryptamine derivatives occur in about equal amounts in the bark while the seed alcaloids dimethyltryptamine and bufotenin were found only in traces.

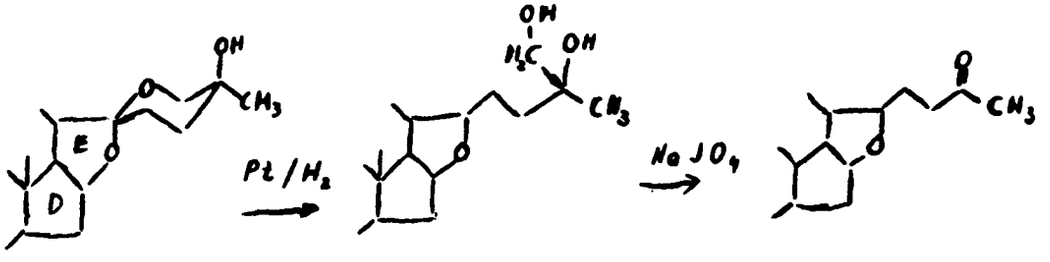
The weakly basic fraction contained small amounts of two bases which give a yellow color with p-dimethylamino benzaldehyde while the tryptamine derivatives react with blue or purple color. Their sensitivity to oxydation caused considerable losses during the isolation procedures so that only qualitative

experiments could be made with the material available. One compound could be isolated as a yellow oxalate. Investigation of UV- and IR spectra indicated the presence of an oxindole system, which also occurs naturally in certain of the more complicated indole alcaloids. Chemical reactions with model compounds (Oxindole, 3-propyl-oindole, 3-propylidene oxindole) did not show however the reactions given by the natural products (fast appearance of an yellow color with p-dimethyl-amino benzaldehyde/HCl, grey-blue coloration after oxydation with $KMnO_4$ or $K_3Fe(CN)_6$ and treatment with acid). These color reactions suggested the presence of an aromatic o-amino ketone similar to kynurenine  which is biologically formed from tryptophane by cleavage of the pyrrole nucleus and which also seemed to be compatible with the UV-spectrum. The IR-spectra of model compounds (o-amino acetophenone, o-amino propiophenone) were however distinctly different from those of the piptadenia bases, especially in the carbonyl region and there were also some differences in the behavior towards oxydizing agents. Another possibility suggested by the UV-spectrum was the presence of an indoline system. While the condensed indoline alcaloids like aspidospermine, ajmaline, and physostigmine do not react with p-dimethyl-aminobenzaldehyde all of the above mentioned color reactions were given by indoline itself. A strong band in the IR-spectrum of the piptadenia base at 1685 cm^{-1} not found in the indoline spectrum might be caused by an amide group in the side chain.

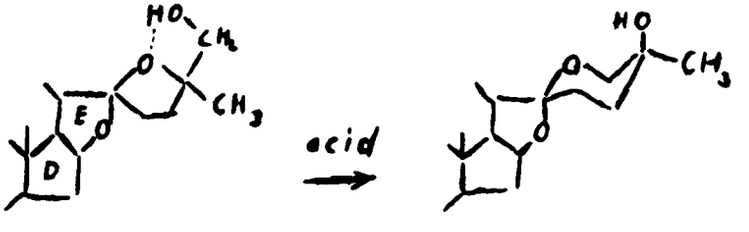
5-Methoxy-N-methyltryptamine and 5-methoxy-N,N-dimethyl-tryptamine methiodide were pharmacologically screened by the Chemical Research Division, Army Chemical Center, Md. LD_{50} with mice was 56 mg/kg and 18 mg/kg respectively (intravenous injection). At non lethal doses both compounds caused decreased activity, vasodilation, low carriage and the former tremors and spansms.

S o l a n u m s i s i i m b r i f o l i u m (solanaceae)
(see previous FTR. p.[7])

The root saponin of this plant had been hydrolysed to give sapogenins which were shown to belong to the spirostanol type by degradation to $\Delta^{5,16}$-pregnadien-3ß-ol-20ene. One hydroxyl-group in the main sapogenin $C_{27}H_{42}O_4$ (I) is thus located in 3 and the other one in ring E or F of the spirostanol system. Its location at $C_{(25)}$ could be proved by hydrogenolytic cleavage of ring F, removal of the hydroxy methyl group by periodate cleavage and comparison of the resulting methyl ketone with a ketone obtained from tigogenin by hydrogenolytic cleavage of ring F, conversion of the $C_{(25)}$ hydroxymethyl group to a methylene group and cleavage with OsO_4 and $NaJO_4$.



Two more sapogenins (II and III) were isolated pure which had the formula $C_{27}H_{40}O_3$ and which contained a diene system. They are probably 3-anhydro sapogenins. One of them had a tertiary hydroxyl group and had the same infrared absorption in the spirostanol region as had I. The other one (III) had a primary hydroxyl group forming an intramolecular hydrogen bond. By treatment with dilute acids III was converted into II. By comparison with authentic material (reineckiagenin and iso-reineckiagenin) it could be shown that the relationship between II and III is as in choligenin and isocholigenin:



Finally the choligenin analogue of Sapogenin I has also been isolated from the hydrolysate. As both dehydration and choligenin -isocholigenin transformation are acid catalysed reactions,

it is most likely that this last mentioned sapogenin is the only original genin from which the others are formed during hydrolysis.

Symplocos celastrinea (symplocaceae)
(see previous FTR p.8)

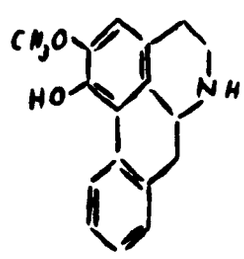
Several alcaloids of the aporphine type had been found in the bark of this tree.

Alcaloid A, m.p. 122-3, $C_{19}H_{21}O_4N \cdot CH_3OH$, could be converted to (+)-Glaucine by methylation of its two free hydroxyl groups. As the two OH-groups could be shown to be located in two different rings and as the physical data of the mono-methyl derivative were similar to those of thalicmidine the structure of a 3,6-dihydroxy-2,5-dimethoxy-aporphine was proposed. Further experiments however could not confirm this structure. A direct comparison of the methochloride of alcaloid A with an authentic sample of laurifolin chloride showed that alcaloid A is 2,5-dihydroxy-3,6-dimethoxy-aporphin.

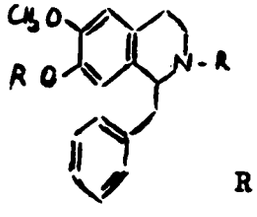
Alcaloid C, m.p. 207-8, $C_{17}H_{17}O_2N$, had been found to be a mono hydroxy, monomethoxy, N-desmethyl aporphine. The free hydroxyl group could be methylated with diazomethane only with difficulty. Much easier methylation occurred in the presence of CO_2 which had been added to minimize oxydation. The resulting product was however the O-methoxy-N-carbomethoxy derivative.

From the optical rotation and the UV-spectrum it could be inferred that both oxygen functions should be in Ring A of the aporphine skeleton and more specifically in position 5 and 6. The position of the hydroxyl group was assumed to be 5 on account of its difficult reaction with diazomethane and from the position of the CH_3-O- signal in the NMR-spectrum.

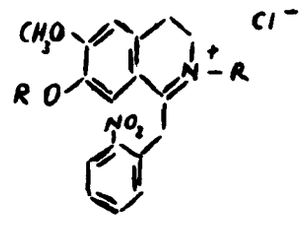
Alcaloid C



Its synthesis was attempted by the following route: O-benzylvanilline was condensed with nitromethane to give ω-nitro-3-methoxy-4-benzyloxy-styrene. This was reduced to the corresponding phenyl ethyl amine with $LiAlH_4$ which was then acylated with o-nitrophenyl acetyl chloride. The yields in the reduction step and the acylation step were only fair or poor, especially the latter because o-nitrophenyl acetyl chloride cannot be isolated pure and the reaction is difficult to control. Cyclisation of the amide with PCl_5 in ether afforded the benzyl dihydrosioquinolinium salt I, which was reduced to the o-Aminobenzyl tetrahydro isoquinoline. An attempt to effect the cyclisation to the aporphine by diazotation and heating in the presence of cupous chloride gave as the only isolable compound a substance which is probably the deamination product II. Further experiments to effect the cyclisation under different conditions will be made.



R = benzyl



II

I

Alcaloid A was pharmacologically screened by the Chemical Research Division, Army Chemical Center, Md. The lethal dose was above 100 mg/kg i.v. Observed effects were decreased activity, prostration, and eyelid ptosis.

Asclepias mellodora (asclepiadaceae)

This plant was collected in Argentina and reported to be toxic. It was extracted with methanol and the concentrated extract partitioned between 50 % methanol and chloroform, after lipids had been removed with benzene. The chloroform soluble part gave a strongly positive test for deoxy sugars and on paper chromatograms several substances were detected

which gave a positive reaction with Kedde's reagent for heart poisons of the cardenolid type. As the occurrence of cardenolides in this plant family is well known and as this group of substances has already been very closely investigated further studies were postponed at present.

Proposals for future work

It is planned to continue the research work on the following subjects which could not be completed in the period covered by this report: skin irritating material and compound A from Daphne mezereum, structure of genin D and of unknown sugars from Ipomoea fistulosa, synthesis of alcaloid C from Symplocos celastrinea.

Contacts have been made with an Indian Institution and with a botanist at the Ivory Coast for the collection of plant material. Some plants have already been received in connection with another research project and we expect to receive more material suitable for the inclusion in this program in the future.

Administrative

The principal investigator under this contract was Prof.Dr.R.Tschesche. Assistant investigator was Dr.Günter Legler.

Expenses

The following is a brief unofficial summary of the number of manhours expended on the contract together with a breakdown of the costs of carrying out the contract.

	cost Deutsche Mark
Principal investigator (25% of his time)	nil
Assistant investigator (100% of his time during 12 months)	15.000.
Technical assistant (100% of her time during 12 months)	7.200.
Technical assistant (100% of his time during 8 months)	4.800.
Expendable supplies (chemicals, glassware)	23.000.
Plant material, air fright	2.000.
	52.000.

No important property was aquired during the contra t period at direct contract expense.